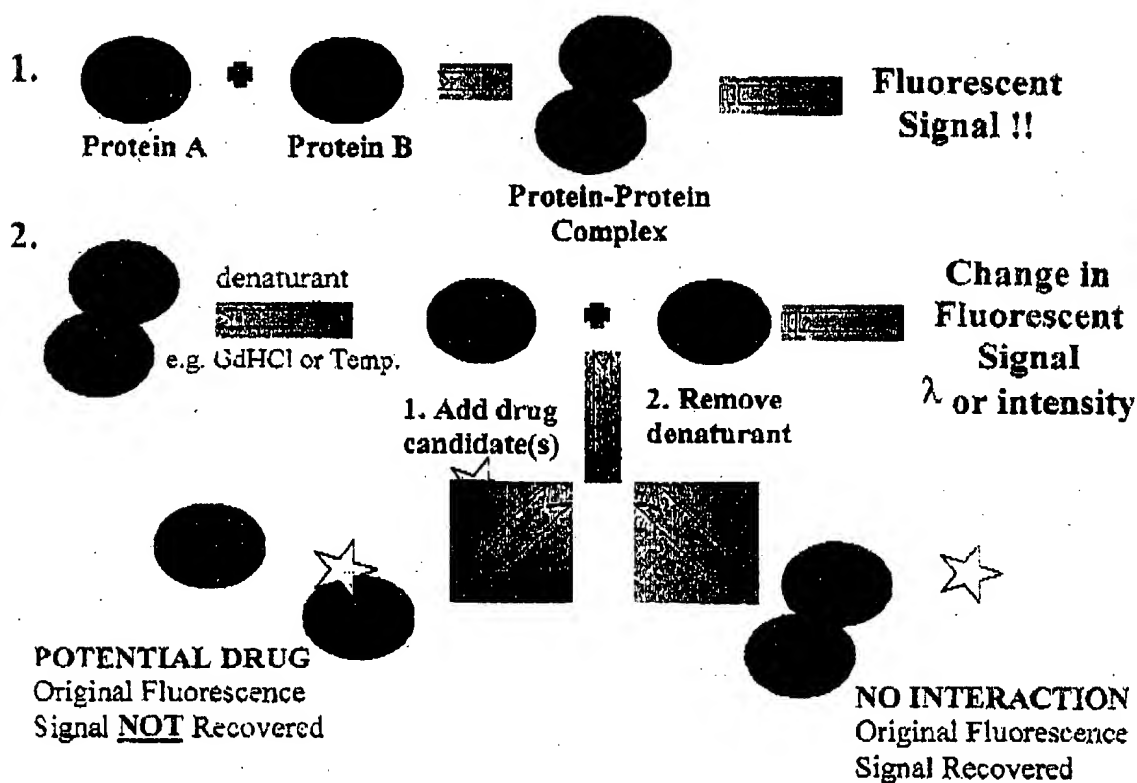


FIGURE 1

Proposed Assay

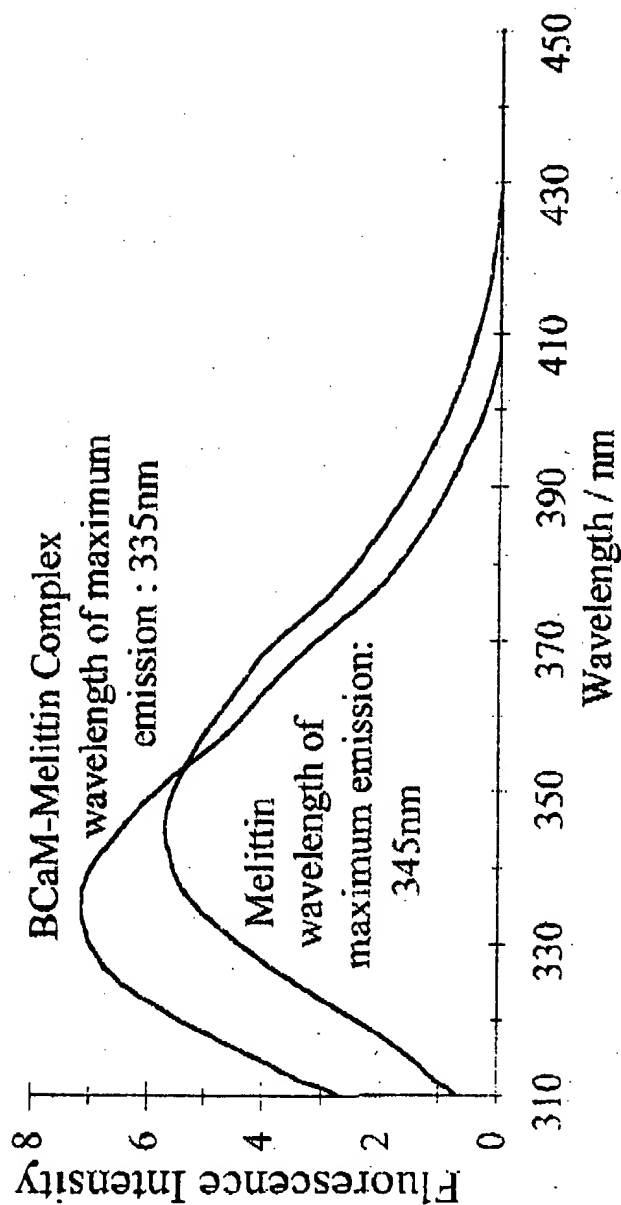


ENTRAP PROTEIN-PROTEIN COMPLEX IN A SOL-GEL DERIVED GLASS

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FIGURE 2

Fluorescence Signal from BCaM-Melittin System



FIGURES 3

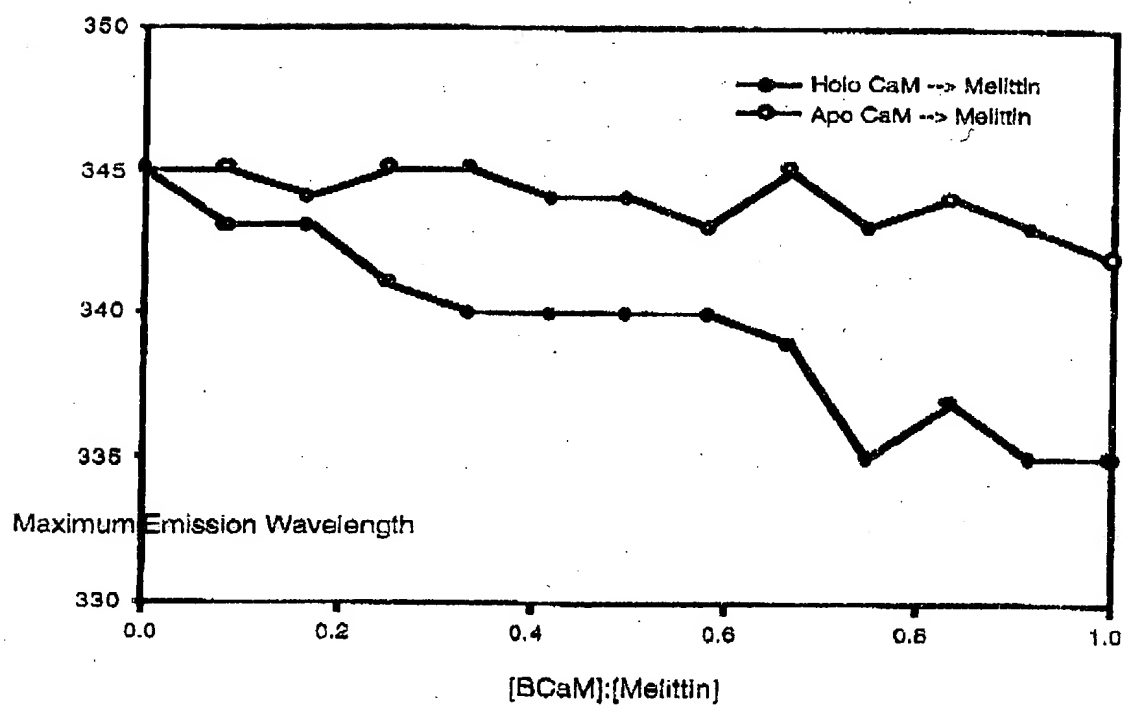
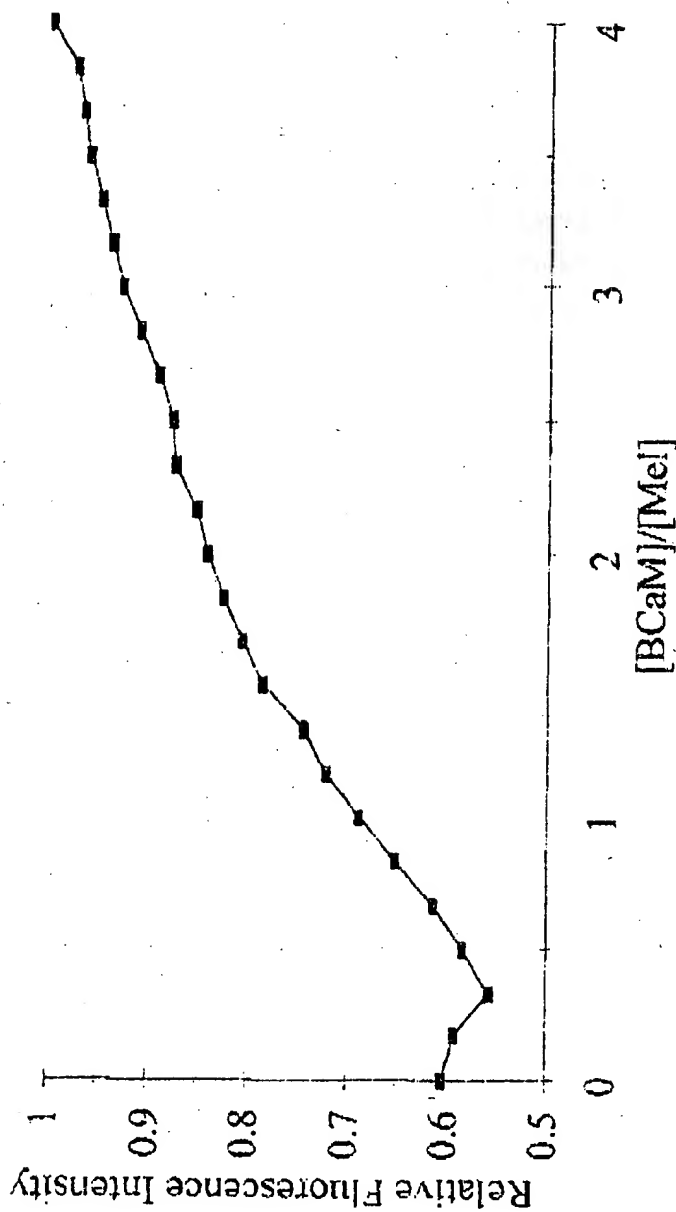


FIGURE 4



The Sol-Gel Process

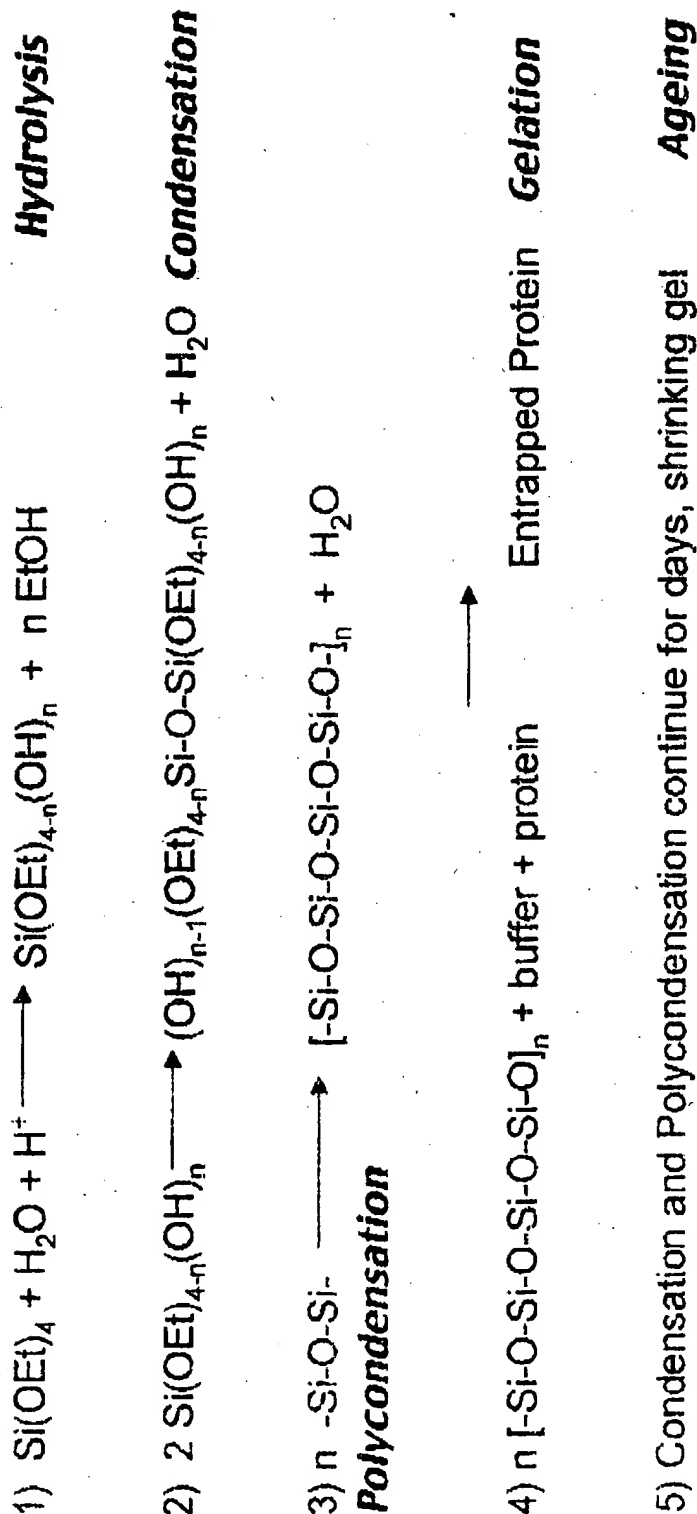
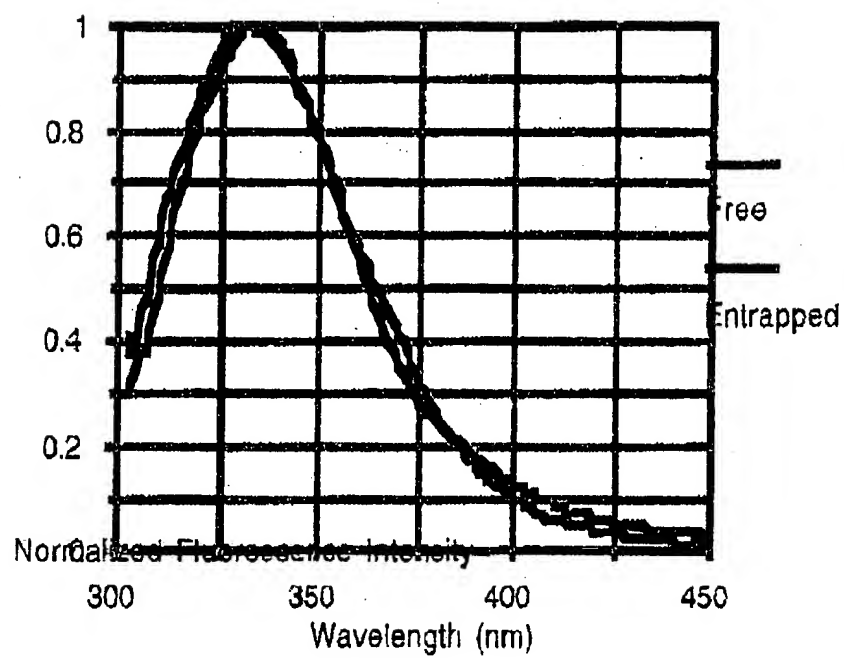


FIGURE 5

FIGURE 6



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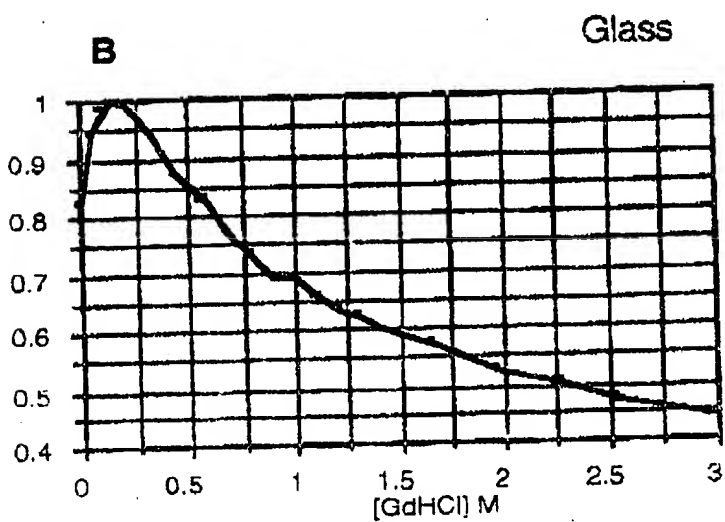
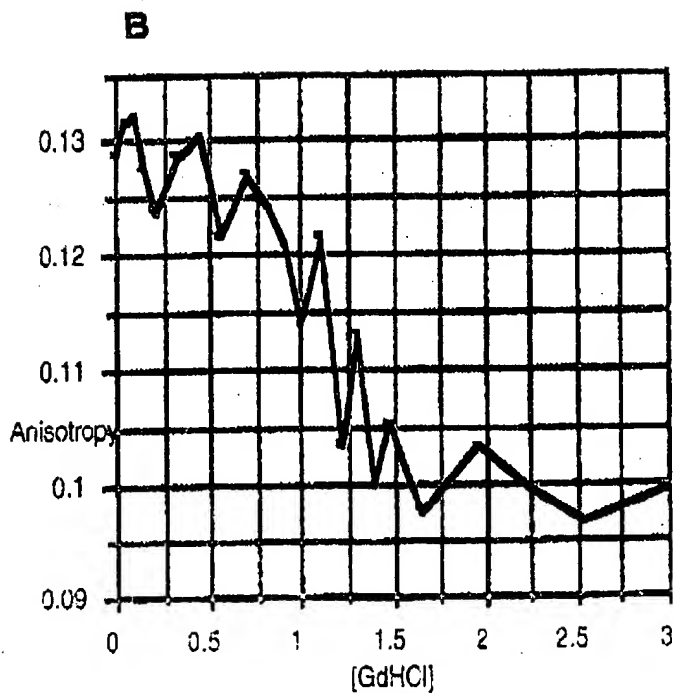
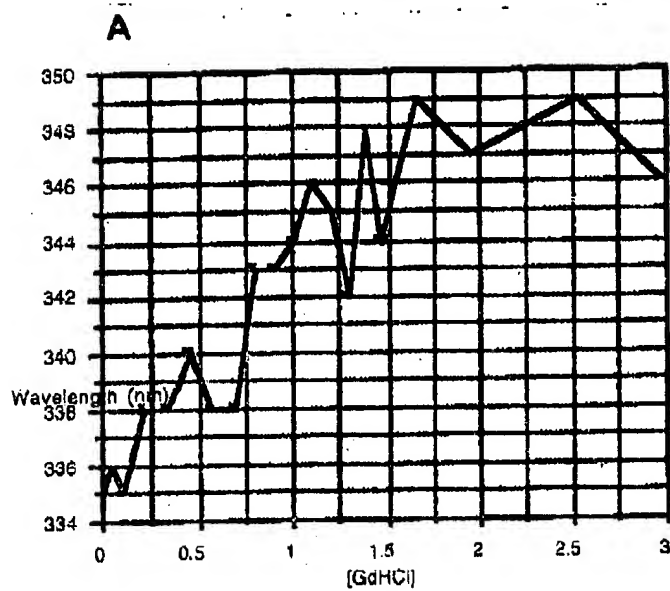


FIGURE 8A-B



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Reversibility of Denaturation for an Entrapped Protein

FIGURE 9

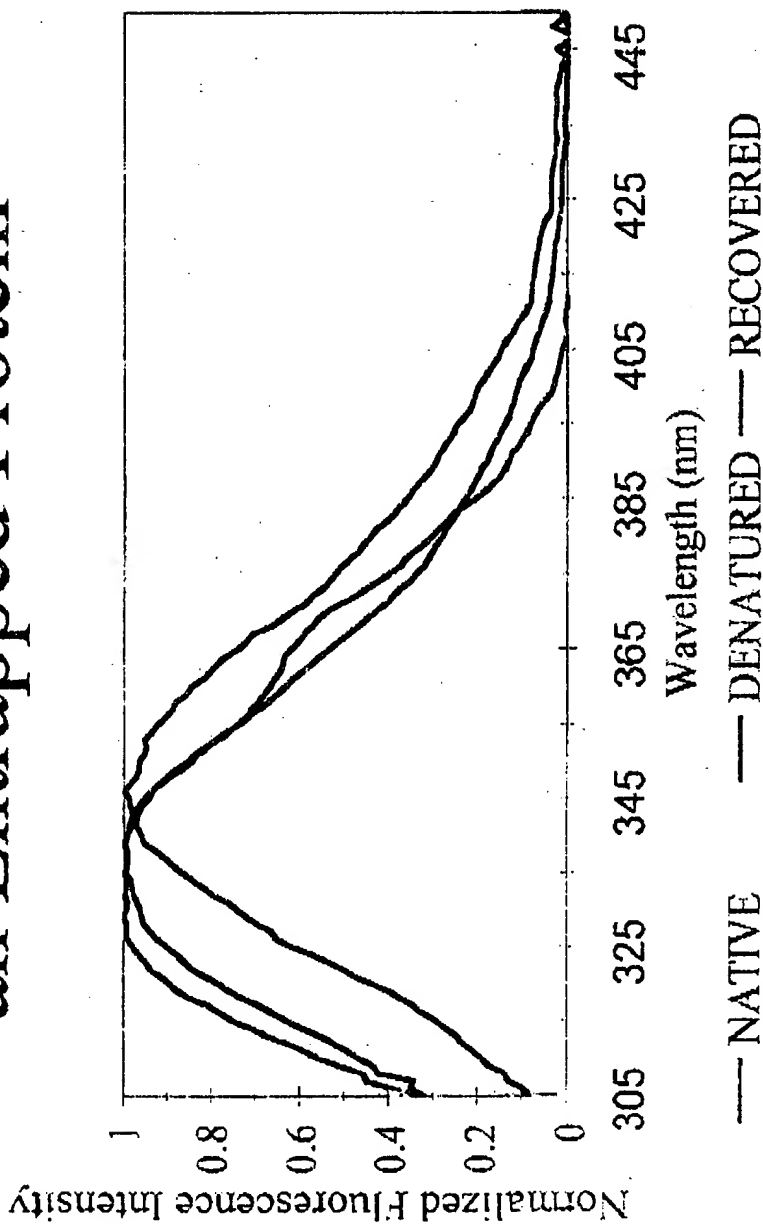


FIGURE 10A-B

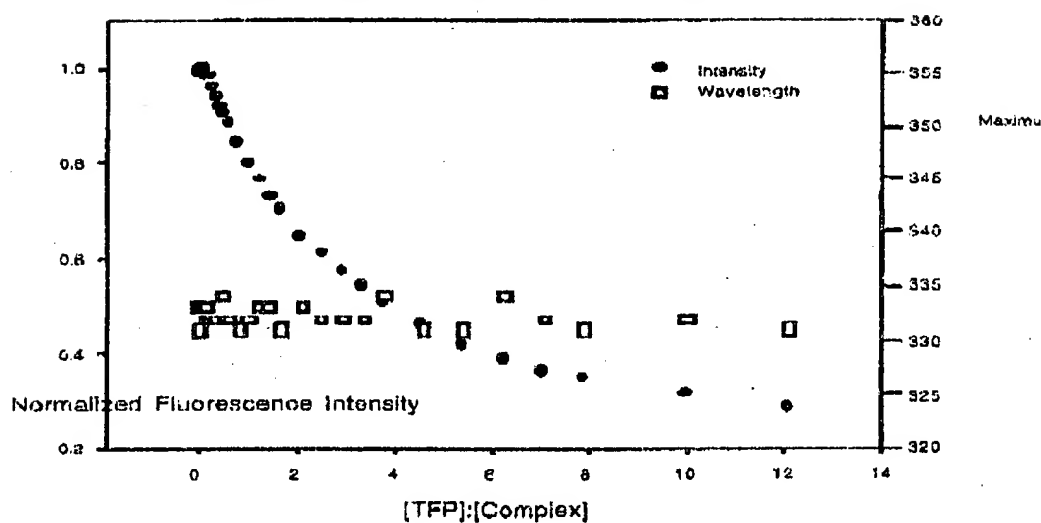
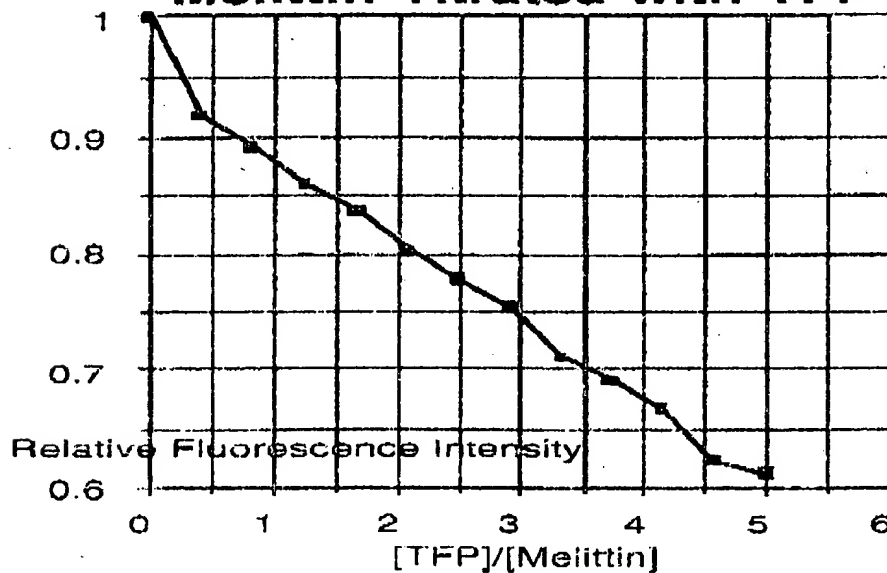
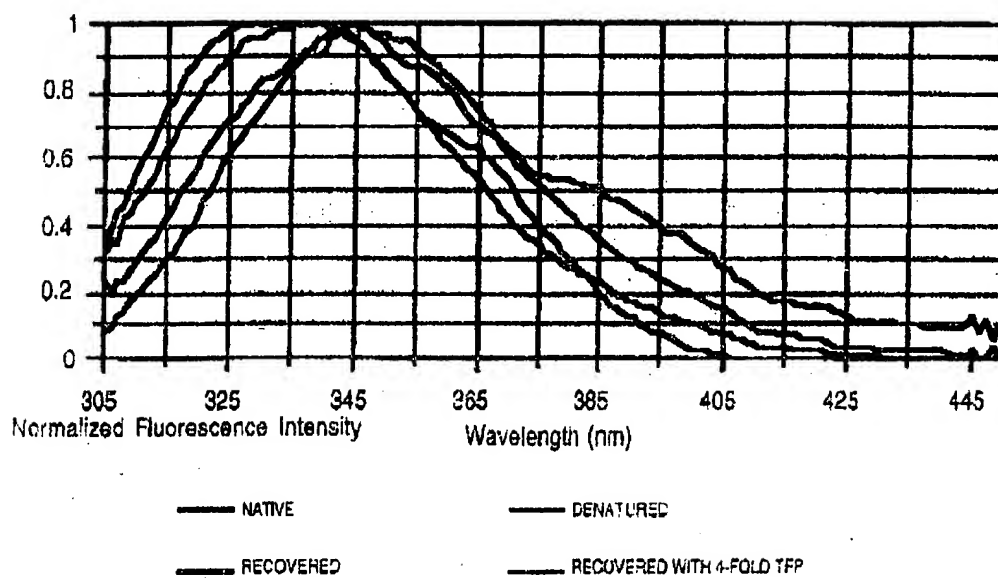
A TFP Added to BCaM-Melittin Complex
Excited at 295 nm, in the Presence of Ca^{2+} **B** Melittin Titrated with TFP

FIGURE 11



THE UNIVERSITY OF CHICAGO

Proof of Concept of the Assay

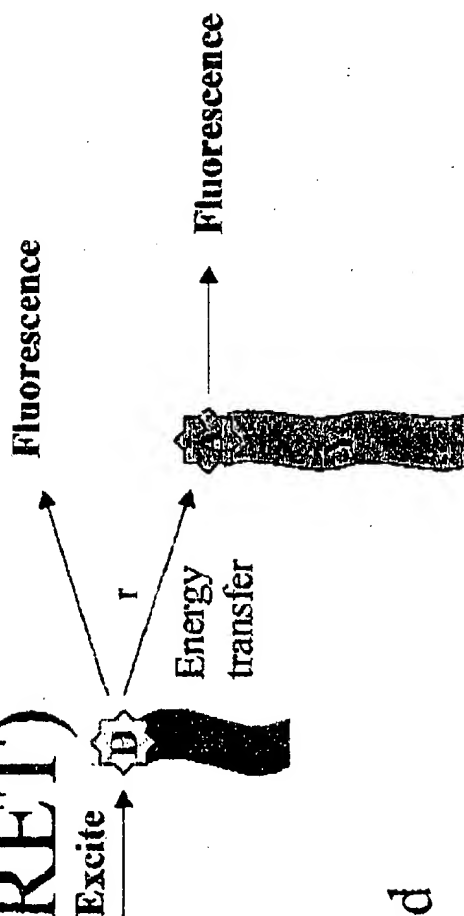
FIGURE 12

Solution	Anisotropy	Wavelength (nm)
Native	0.127	335
Denatured	0.129	334
Recovered	0.099	346
Denatured	0.136	331
Recovered with TFP	0.115	346
	0.121	346

FIGURE 13

Florescence Resonance Energy

Transfer (FRET)



Donor excited

Emits at λ_D that overlaps Acceptor absorbance

Acceptor excited and emits at a longer λ_A

Efficiency of energy transfer dependent on distance between donor and acceptor

Förster distance (R_0) = 50% efficiency

FIGURE 14

**Donor attached to Protein A, Acceptor
attached to Protein B**

Efficiency of energy transfer (E) related to
distance (r) by;
$$\frac{R_0^6}{R_0^6 + r^6}$$

- Use donor molecule which absorbs at longer λ to TFP \therefore avoid exciting TFP

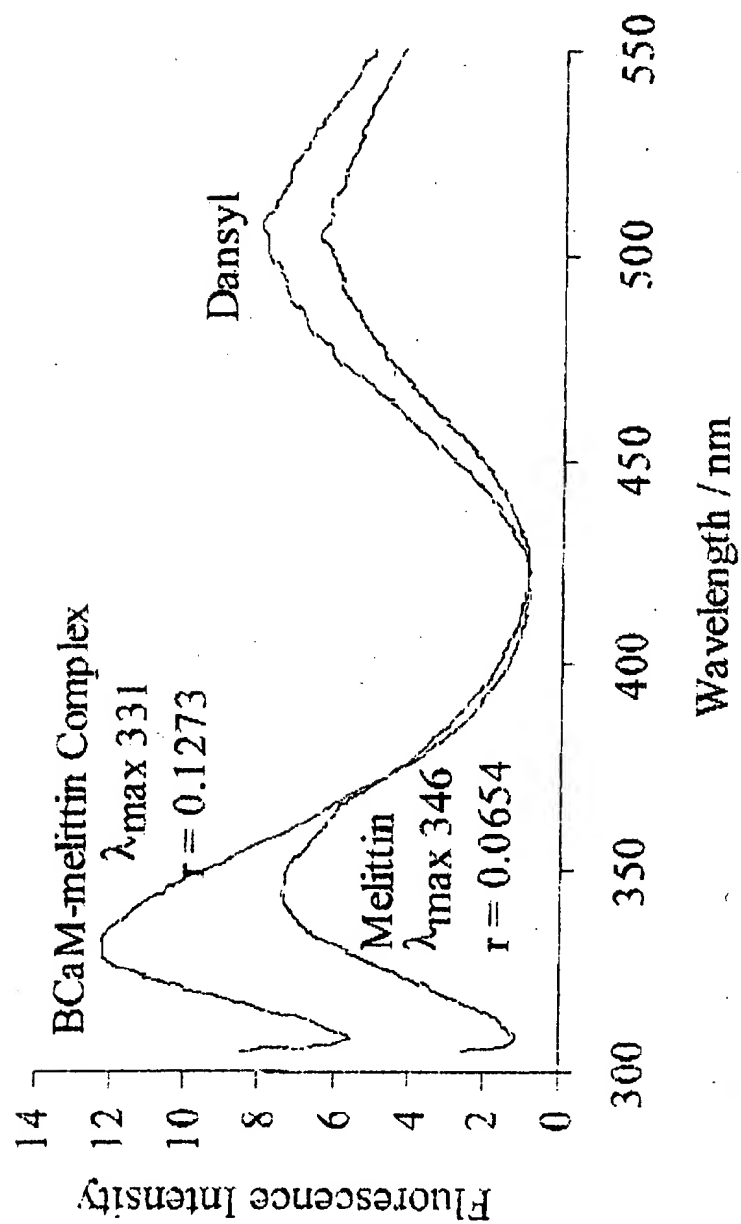
FIGURE 15

MelittinBCaM

- | | |
|-------------------|--------------------------|
| Energy Donor | • Energy Acceptor |
| • Dansyl Chloride | • Fluorescein derivative |
| • Absorbs @ 370nm | • Absorbs @ 494nm |
| • Emits @ 500nm | • Emits @ 518nm |

$$R_0 = 3.3 - 4.1 \text{ nm}$$

FIGURE 16



Dansyl doesn't interfere with PP interaction

FIGURE 17

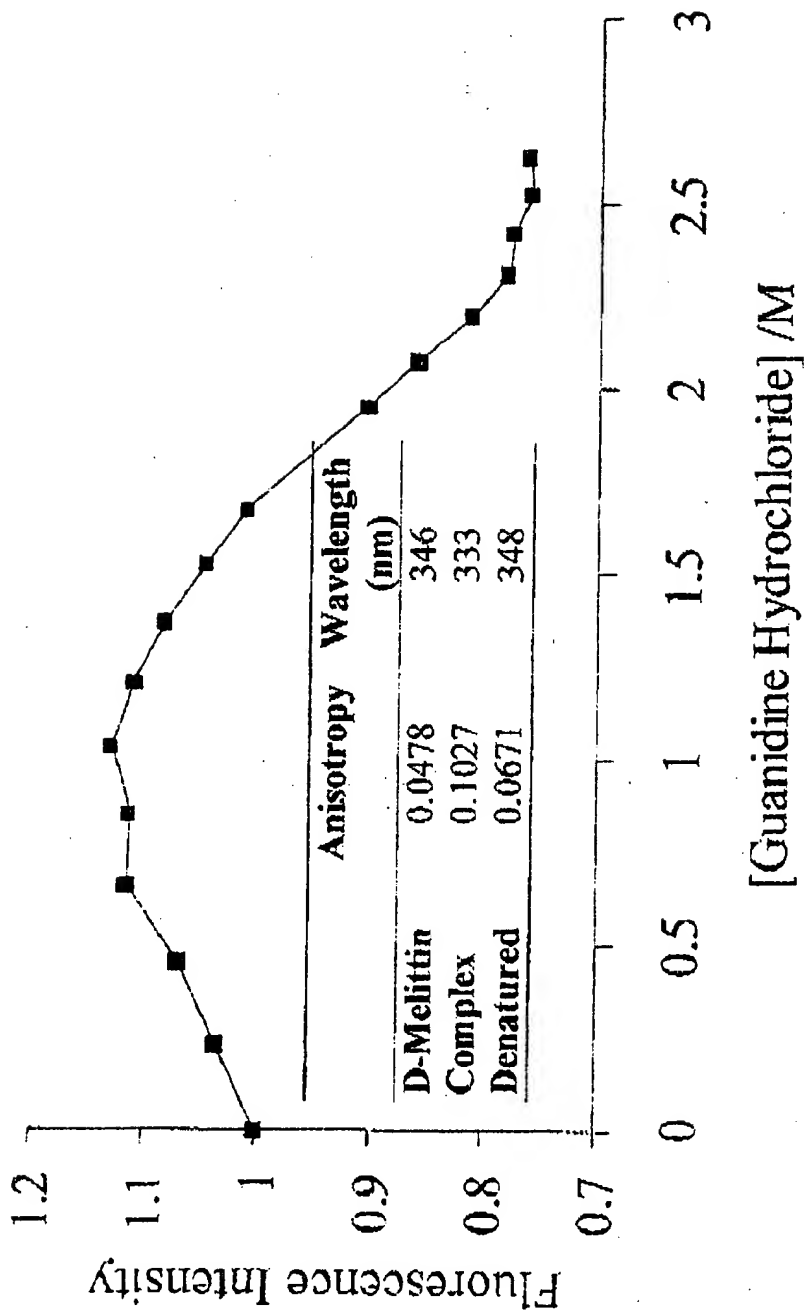
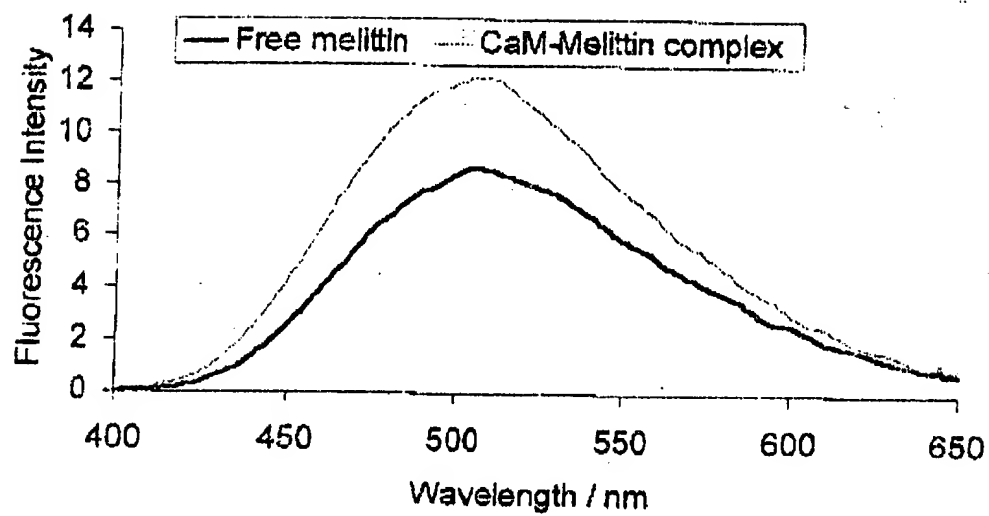
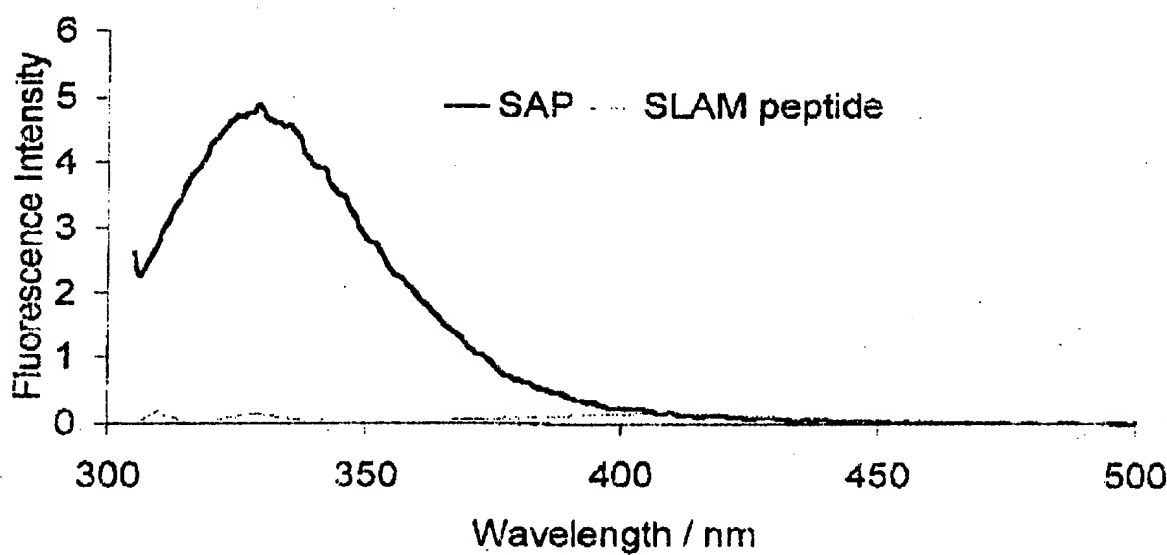


FIGURE 18



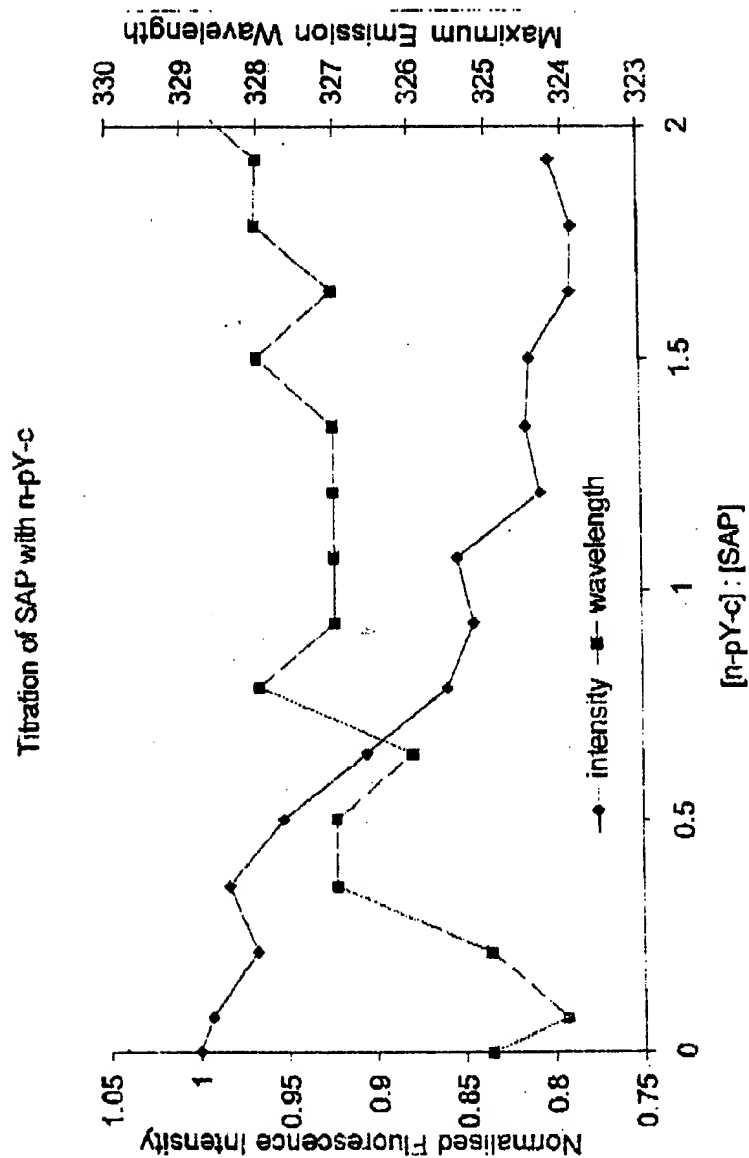
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FIGURE 19

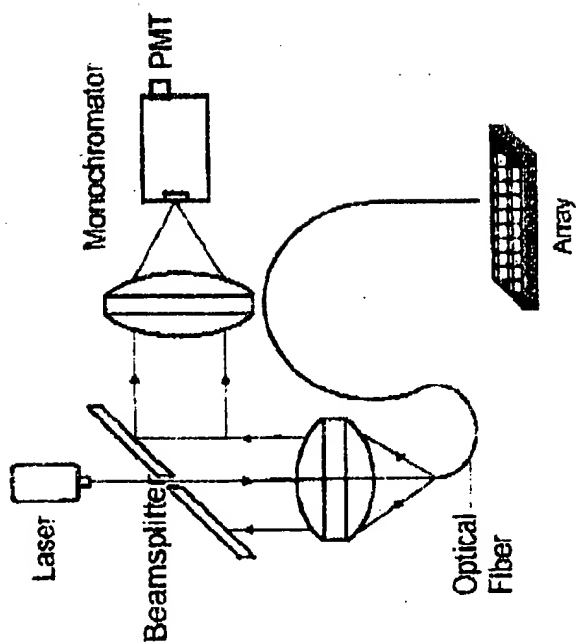
Fluorescence Spectra of SAP and Phosphorylated
SLAM Peptide

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FIGURE 20



A high-contrast, black and white photograph showing a person's legs and feet. The person appears to be standing or walking, with their legs and feet visible against a dark, textured background. The image is grainy and has a high level of contrast, with the legs and feet appearing as bright white shapes against the dark background. The person is wearing a shoe, which is visible as a bright white shape on the right side of the image. The overall image has a grainy, high-contrast quality, similar to a photocopy or a high-contrast scan of a photograph.



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1 2 3 4 5 6

1 2 3 4 5 6 7 8 9

$2 \times 10^{-4} \text{ M}$
 $2 \times 10^{-5} \text{ M}$
 $3 \times 10^{-6} \text{ M}$
 $6 \times 10^{-8} \text{ M}$
 $8 \times 10^{-9} \text{ M}$
 $2 \times 10^{-9} \text{ M}$
 $2 \times 10^{-10} \text{ M}$
 $2 \times 10^{-11} \text{ M}$
 $2 \times 10^{-12} \text{ M}$

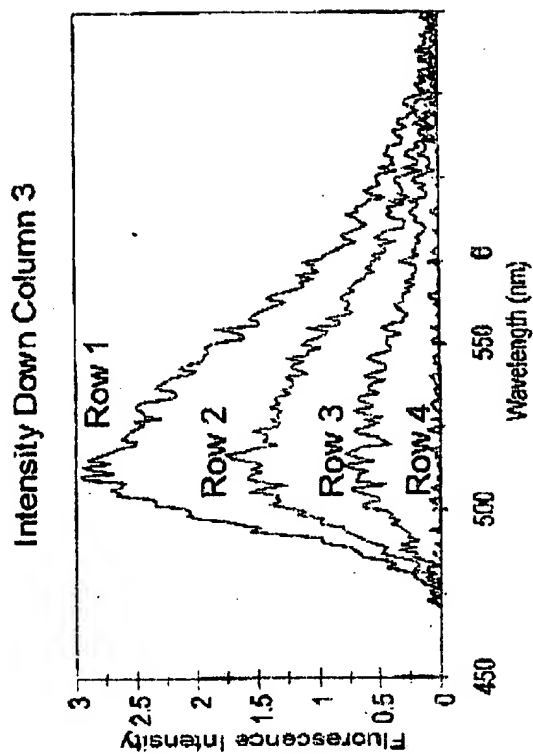
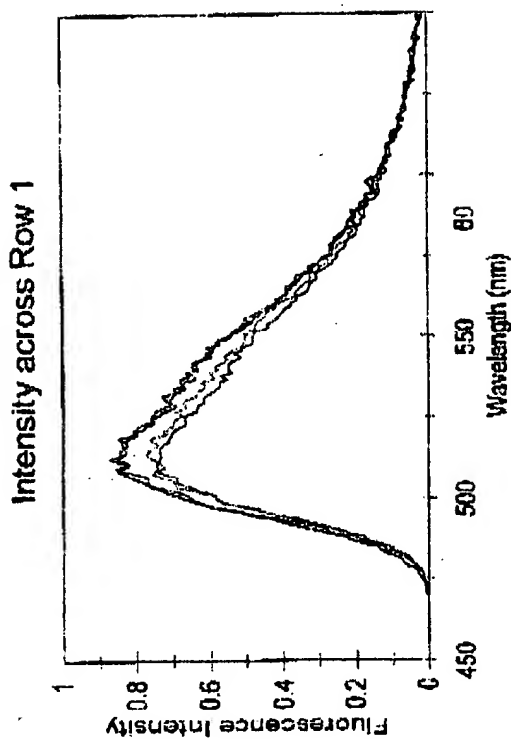


FIGURE 22